

Cloning and characterization of the rad4 gene of *Schizosaccharomyces pombe*; a gene showing short regions of sequence similarity to the human XRCC1 gene

Article (Published Version)

Fenech, M, Carr, A M, Murray, J, Watts, F Z and Lehmann, A R (1991) Cloning and characterization of the rad4 gene of *Schizosaccharomyces pombe*; a gene showing short regions of sequence similarity to the human XRCC1 gene. *Nucleic Acids Research*, 19 (24). pp. 6737-6741. ISSN 0305-1048

This version is available from Sussex Research Online: <http://sro.sussex.ac.uk/id/eprint/16932/>

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the URL above for details on accessing the published version.

Copyright and reuse:

Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

Cloning and characterization of the *rad4* gene of *Schizosaccharomyces pombe*; a gene showing short regions of sequence similarity to the human *XRCC1* gene

M.Fenech⁺, A.M.Carr, J.Murray¹, F.Z.Watts¹ and A.R.Lehmann*

MRC Cell Mutation Unit and ¹Microbial Genetics Laboratory, School of Biology, University of Sussex, Falmer, Brighton BN1 9RR, UK

Received October 16, 1991; Revised and Accepted November 21, 1991

EMBL accession no. X62676

ABSTRACT

The *rad4.116* mutant of the fission yeast *Schizosaccharomyces pombe* is temperature-sensitive for growth, as well as being sensitive to the killing actions of both ultraviolet light and ionizing radiation. We have cloned the *rad4* gene by complementation of the temperature sensitive phenotype of the *rad4.116* mutant with a *S. pombe* gene bank. The *rad4* gene fully complemented the UV sensitivity of the *rad4.116* mutant. The gene is predicted to encode a protein of 579 amino acids with a basic tail, a possible zinc finger and a nuclear location signal. The amino terminal part of the predicted *rad4* ORF contains two short regions of similarity to the C-terminal part of the human *XRCC1* gene. Codon usage suggests that the gene is very poorly expressed, and this was confirmed by RNA studies. Gene disruption showed that the *rad4* gene was essential for the mitotic growth of *S.pombe*.

INTRODUCTION

The existence of several human genetic disorders in which defective DNA repair is associated with severe and diverse clinical symptoms attests to the importance of DNA repair for the maintenance of a healthy condition in man (1). Biochemical, cellular and molecular studies in prokaryotic and eukaryotic organisms have demonstrated the enormous complexity of DNA repair processes (2). Thus, in the budding yeast, *Saccharomyces cerevisiae*, to date more than 30 complementation groups have been identified as affecting DNA repair (3), and in mammals the number of gene products is anticipated to be over 100. Dissection and understanding of the mechanism of DNA repair will be assisted by the cloning and analysis of DNA repair genes from different organisms, and the characterisation of the functions of the gene products.

In the fission yeast, *Schizosaccharomyces pombe*, twenty-three complementation groups governing the response to UV and/or ionizing radiation have been identified (4, 5). The genes

corresponding to these complementation groups have been designated *rad*. Among the *S. pombe rad* mutants, some were reported to be sensitive to UV alone (e.g. *rad13*), others to γ -rays alone (e.g. *rad22*), but most mutants are sensitive to both UV and γ -radiation (4). Many of the mutants have altered frequencies of recombination or induced mutation (4, 6), but very little biochemical characterization of the mutants has been carried out.

In order to understand the functions of the genes governing the response to radiation in *S. pombe*, we have cloned several of these putative DNA repair genes. In this paper we describe the cloning and characterisation of the *rad4* gene. *rad4.116* mutants are temperature-sensitive for growth, as well as being moderately sensitive to UV, γ -irradiation and ethylmethane-sulfonate (7). All these properties have been shown to result from a single mutation (7). The *rad4.116* mutant has a UV-induced mutation frequency similar to that of wild-type cells (6).

MATERIALS AND METHODS

Strains and media

E. coli strain DH5 α or DH5 α F' were used for all transformations and plasmid maintenance. The *S. pombe* strains used in our experiments are listed in Table 1. Crosses were carried out using the method of Gutz *et al.* (8). The *leu1-32* allele was derived from strain NRC3250, and the *ura4.D18* allele was from SP.011. All strains were routinely maintained on YES (8). Selection for plasmid-containing cells was carried out in minimal medium or on agar plates (9). Minimal agar containing 1.2M sorbitol was used to plate spheroplasts after transformation (10). Minimal medium modified to contain 0.05g/l NH₄Cl was used for mating and sporulation.

Gene banks

The first gene bank used in our experiments was constructed previously (11) from a partial *Hind*III digest of *S. pombe* DNA cloned into the *Hind*III site of the yeast shuttle vector pDB262

* To whom correspondence should be addressed

⁺ Present address: CSIRO Division of Human Nutrition, PO Box 10041 Gouger St, Adelaide 5000, Australia

Table 1. *S. pombe* strains

| | |
|---------|---|
| NRC2342 | <i>rad4.116</i> , <i>h⁻</i> ; from the Culture Collection of the National Research Council (NRC) of Canada via Dr A.Nasim. |
| NRC3250 | <i>leu1.32</i> , <i>ade6.216</i> , <i>ura4</i> , <i>h⁺</i> ; from NRC, Canada. |
| SP.011 | <i>leu1.32</i> , <i>ade6.704</i> , <i>ura4.D18</i> , <i>h⁻</i> ; from P. Nurse, Oxford. |
| SP.012 | <i>leu1.32</i> , <i>ade6.704</i> , <i>ura4.D18</i> , <i>h⁺</i> ; from P. Nurse, Oxford. |
| SP.223 | <i>leu1.32</i> , <i>h⁻</i> |
| SP.557 | <i>rad4.116</i> , <i>leu1.32</i> , <i>h⁺</i> |
| SP.558 | <i>rad4.116</i> , <i>ura4.D18</i> , <i>h⁻</i> |
| SP.507 | <i>leu1.32</i> / <i>leu1.32</i> , <i>ade6.704/ade6.704</i> , <i>ura4.D18/ura4.D18</i> , <i>h⁺/h⁺</i> (diploid) |
| SP.1P | <i>rad4.116</i> , <i>leu1.32</i> , <i>prad4</i> |

(12). The second gene bank was constructed from a partial *Sau* 3A digest of *S. pombe* DNA cloned into the shuttle vector, pUR19 (N.Barbet, W.J.Muriel and A.M.C., manuscript in preparation), which contains the *S. pombe ura4* gene and the *ARS1* sequence inserted at the *Nde* I site of pUC19.

Transformations

E. coli DH5 α or DH5 α F' were transformed with DNA using procedures described by Hanahan (13). *S. pombe* spheroplasts were transformed by the CaCl₂/polyethylene glycol procedure of Beach and Nurse (14).

Instability tests

To show that the *rad 4* complementing activity resided on a transforming plasmid, cells were streaked to single colonies on complex medium. Replicas were then taken on to plates containing either minimal medium without leucine, which were incubated at 30°, or complex medium followed by incubation at 37°. Co-segregation of the two phenotypes indicated that they were coded for by plasmid-borne sequences.

Cell survival

S. pombe cells were serially diluted and 10³–10⁴ cells were plated on minimal medium. The plates were immediately UV-irradiated in a Stratagene 'Stratalinker', and colonies counted after 3 days' incubation at 30°.

DNA extractions

Plasmid DNA preparations were carried out using standard procedures (15). DNA was extracted from *S. pombe* according to the method of Aves *et al.* (16) by lysis with sodium dodecyl sulphate and precipitation with potassium acetate.

RNA extraction and Northern blotting

RNA was extracted from *S. pombe* cells by vortexing with glass beads followed by phenol extraction and ethanol precipitation (17). Poly A⁺-RNA was purified on oligo-dT-cellulose mini-columns (15). For Northern blotting 5 μ g poly A⁺-RNA were glyoxylated and electrophoresed on 1% agarose gels at 45v for 5 hours. After electrophoresis the RNA was transferred to Gene Screen-Plus (New England Nuclear) and hybridized using standard conditions.

cDNA synthesis and polymerase chain reaction (PCR)

Oligonucleotides used were: 1, GGTATCCATTCACTAGACAT; 2, ATGTGTAGTGAATGGATACC; 3, GCAGCCAA-GGATTGACCAGACTT. 10 μ g total *S. pombe* RNA was reverse transcribed in a volume of 20 μ l with avian myeloblastosis virus

reverse transcriptase at 42° using 0.5 μ g oligonucleotide 3. PCR was performed using as template either 10% of the cDNA reaction or 0.1–1 μ g *S. pombe* genomic DNA in a 100 μ l reaction containing 0.6mM dNTPs, 100 pmoles each of oligonucleotides 2 and 3 and 1 Unit *Taq* polymerase (Cetus). The solutions were overlaid with 100 μ l mineral oil and then subjected to 25 cycles of PCR with 1 min at 94° and initially 2 min at 60° and 2 min at 70°, the latter two periods increasing by 3% each cycle. The amplification products were purified using GeneClean (Strathec Scientific Ltd.) and sequenced directly with oligonucleotide 3 as sequencing primer, using the procedure described by Dorado *et al.* (18).

Primer extension

Oligonucleotide 1 was labelled at the 5'-end with [γ -³²P]ATP and polynucleotide kinase. 1 μ l containing 0.6 μ g oligonucleotide was annealed with 3 μ g *S. pombe* poly A⁺-RNA by ethanol precipitation, redissolving in H₂O and heating at 75° for 5 minutes, then allowing to cool for 30 minutes. The annealed mixture was then incubated with Murine Moloney Leukaemia Virus reverse transcriptase for 1 hour at 37°. The product was ethanol precipitated and run on a 6% urea/polyacrylamide gel.

DNA sequencing

DNA fragments to be sequenced were cloned into M13mp18 or mp19. A series of nested deletions were constructed using the exonuclease III/S1 nuclease procedure of Henikoff (19). Sequencing of both strands was effected using the dideoxynucleotide procedure with Sequenase (United States Biochemicals) and ([α -³⁵S] thio) dATP, using the procedures recommended by the manufacturers.

RESULTS

Cloning of the *rad4* gene

Strain NRC3250 containing the *leu1.32* marker was crossed with strain NRC2342 containing the *rad4.116* mutation to produce strain SP.557 (*rad4.116*, *leu1.32*) for use as a recipient for transformation. This strain was transformed with an *S. pombe* gene bank constructed in the yeast shuttle vector pDB262. Transformants were selected for their ability to grow in the absence of leucine. An estimated 24,000 transformants were obtained. After 24 hours incubation at 30°, the plates were shifted up to the non-permissive temperature of 37°. After five days' growth, three colonies survived at 37°, one of which, designated *rad4B9*, was co-unstable for leucine prototrophy and the temperature-resistant phenotype.

Plasmid DNA was extracted from these complemented cells and transformed into *E. coli* strain DH5 α . Transformants, selected in tetracycline, contained a plasmid which was designated *prad4*. Retransformation of strain SP.557 (*rad4.116*, *leu1.32*) with this plasmid corrected the temperature sensitivity of the mutant.

The complemented SP.1P strain was grown in rich medium and analysed for instability as described above. Three colonies with stable temperature-resistance and uracil prototrophy were presumed to have integrated the plasmid at the *rad4* locus. In order to check this, DNA was isolated from the integrants, digested with different restriction enzymes and hybridized with the insert from *prad4* following electrophoresis and Southern transfer (not shown). In all cases the *rad4* banding pattern of the integrants was altered when compared with the wild-type

strains and strains carrying the *rad4.116* mutations, confirming that the plasmid had integrated at the expected position. Two of the integrants were then crossed with strain SP.223 (*h⁻, leu 1.32*). After sporulation, only 3 out of 800 progeny were temperature-sensitive, confirming that the plasmid had integrated at the *rad4* locus.

Characterisation of the *rad 4* gene

A restriction map of plasmid *prad4* is shown in Figure 1a. The 2.67Kb *Hind* III insert was cloned into M13mp19 in both orientations and sequenced in both directions using the exonuclease deletion procedure (19). The sequence (see Figure 2) revealed an open reading frame starting at base number 1045 and extending to the *Hind* III site at base number 2665 (H2 in Figure 1) at the end of the insert. This suggested that the insert contained a truncated form of the gene lacking the C-terminal end. Subsequently a new *S. pombe* gene bank was constructed in an improved *S. pombe* vector pUR19 (N. Barbet, W. J. Muriel and A. M. C., manuscript in preparation). A plasmid, *praf9* correcting the temperature-sensitive phenotype of the *rad4.116* mutant was isolated from this gene bank by functional complementation of the strain SP.558 (*rad4.116, ura4.D18*) and a restriction map is shown in Figure 1b. The map correlated perfectly with that of the original *prad4* plasmid and in addition it extended further in both directions. Sequencing revealed a termination codon approximately 115 base pairs from H2. Plasmid *praf9* therefore contains the complete ORF as shown.

Analysis of the DNA sequence of the *rad4* gene (EMBL Accession No. X62676) predicts a protein of 579 amino acids with a calculated molecular weight of 66580. 34% of the amino acids are hydrophobic and 28% are hydrophilic. 12% of the amino acids are strongly basic and 13% are acidic, with a calculated pI of 6.9. The principal features are shown in Figure 2. There is a basic tail (RKLRRR). Also six out of eight amino acids at positions 173–180 (KRGKKRDR) are strongly basic. Both these sequences contain putative nuclear location signals (NLS), which closely match the NLS consensus sequence, K/R K/RXX/R. Starting at amino acid 36 there is a CysXXCysX₁₈HisX₄Cys, which may represent a zinc finger. Codon usage (20) suggests that the gene is poorly expressed. This was confirmed in RNA studies described below.

Scanning of the Genbank, EMBL and PIR data bases using the DNASTAR programmes and TFASTA at Daresbury laboratory revealed that two short regions in the N-terminal half of the *rad4* gene product had strong sequence similarity to two regions in the C-terminal half of the human *XRCC1* gene product (21) (see Figure 2). These two regions comprise a stretch of 11 amino acids, of which 9 are identical between the yeast and human genes (aa 64–75 of *rad4*: PDLTRDVTHLI and aa 351–361 of *XRCC1*: PDWTRDSTHLI), and another stretch of 7 amino acids, of which 6 are identical (aa239–245 of *rad4*: FYLYEFP and aa 548–555 of *XRCC1*: FFLYGEFP).

UV survival

Plasmid *praf9* containing the complete ORF was re-introduced by transformation into strain SP.558 (*rad4.116, ura4.D18*). Uracil-independent transformants were picked and instability testing showed that they contained plasmids which corrected the temperature sensitivity of the *rad4.116* mutant. The response of these transformants to UV-irradiation is shown in Figure 3. Whereas *praf9* completely restored the UV-response of strain SP.558 to that of wild-type *S. pombe*, transformants carrying

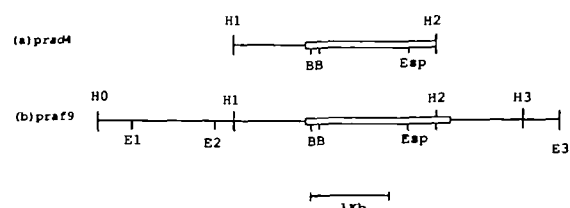


Figure 1. Restriction maps of inserts in plasmids containing the *rad4* gene. (a) *prad4* containing a 2.67Kb *Hind* III insert lacking the C-terminal 37 amino acids of the putative *rad4* ORF. (b) *praf9* containing the whole putative *rad4* ORF (denoted by a bold line). B, *Bgl* II; E, *Eco*RI; Esp, *Esp*I; H, *Hind*III.

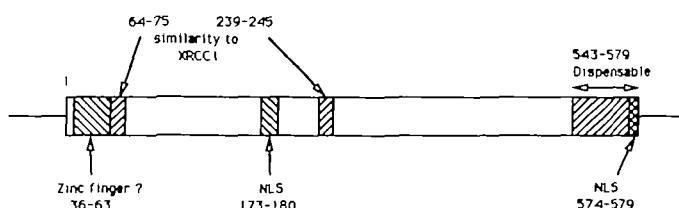


Figure 2. Features of the *rad4* protein.

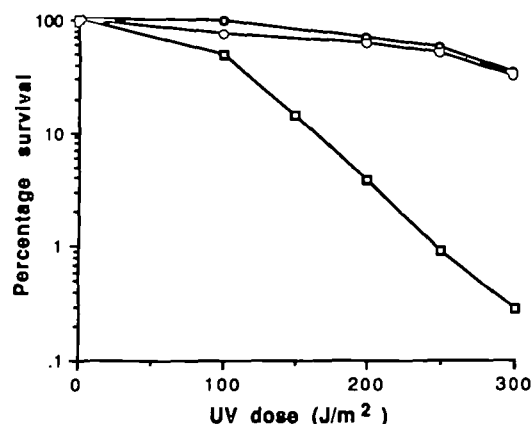


Figure 3. UV survival. Strain SP.558 (*rad4.116, ura4*) transformed with the complementing plasmid *praf9* (○) or the vector pUR18 (□), and the strain SP.011 (*rad4⁺*) transformed with the vector pUR19 (◇) were plated out at about 10³ cells per plate on minimal medium lacking uracil, UV-irradiated, and colonies counted after 3 days at 30°.

the pUR19 vector alone did not become more resistant to UV-irradiation.

Expression of the gene

Northern blots hybridized with the *rad4* gene showed a mRNA species of approximately 1.9kb (Figure 4). A signal was obtained only with 5μg polyA⁺-RNA, demonstrating that the gene was poorly expressed, in accordance with the prediction from codon usage. Primer extension experiments (not shown) revealed a transcription start site approximately 90bp upstream from the ATG at the beginning of the open reading frame.

The DNA sequence showed a potential intron near the amino-terminal end of the gene with splicing consensus sequences similar to those described by Russell (20) (nucleotides 1425–1487). In

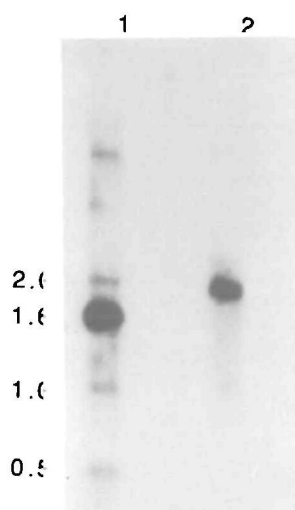


Figure 4. Autoradiogram of Northern blot of *rad4*. 5 μ g poly A⁺-RNA were electrophoresed in a glyoxal-agarose gel, transferred to a nylon filter and hybridized with the 1.6Kb *Bgl* II–*Hind*III fragment of *prad4* (see Figure 1a), which contained most of the *rad4* ORF. Lane 1, molecular weight marker- λ DNA digested with *Pst*I; lane 2, RNA from *S. pombe*.

order to determine whether this putative intron was spliced out of the RNA, PCR was carried out using either *S. pombe* genomic DNA or cDNA as template and primers 2 and 3 (see MATERIALS AND METHODS) which spanned the region from nucleotide 1045 (the start of the ORF) to 1523. The resulting 480bp fragments were identical in size whether the cDNA or genomic DNA was used as template, and sequencing of the first 100 bases of the amplification products revealed no differences between the two samples (unpublished observations). Treatment of the RNA, from which the cDNA was synthesized, with RNase prior to cDNA synthesis, abolished the subsequent production of PCR product from this sample, confirming that the PCR product from the cDNA sample did indeed result from using cDNA as substrate, rather than contaminating genomic DNA. These data indicate that the putative splice sites are not generally utilised in the transcription of the *rad4* gene.

Gene disruption

The 4.4kb *Eco* R1 fragment (E2-E3) from praf 9 (Figure 1b) containing the whole of the *rad4* coding region was subcloned into pUC9. The 1.3kb *Bgl* II–*Esp*I fragment from praf9 containing a large part of the *rad4* coding region (see Figure 1) was excised and replaced with a *Sph*I linker. A 1.7kb *Sph*I-linked fragment containing the *S. pombe ura4* gene (22) was inserted into the engineered *Sph*I site in the *rad4* gene to produce a disrupted gene, which was then excised from the plasmid as a 4.8kb *Eco*R1 fragment and transformed into diploid *S. pombe* strain SP.507 carrying the *ade6.704*, *leu1.32*, *ura 4.D18*, *h⁺* alleles. Stable *ura⁺* transformants were isolated. Southern blotting of DNA from two stable diploid transformants following *Hind*III digestion showed, in addition to the 2.7kb *Hind*III fragment of the wild-type *S. pombe* chromosome, the expected 3.1kb fragment containing the disrupted gene (not shown), confirming that these transformants had integrated the disrupted gene. One of the integrant strains was streaked out on rich medium. Colonies were replica plated onto low nitrogen plates, incubated at 30° for two days and exposed to iodine vapour.

Darkly staining revertant colonies (assumed to be *h⁺/h⁹⁰*) were then recovered. When they were induced to sporulate, no *ura⁺* colonies were obtained, demonstrating that the *rad4* gene was essential for survival of *S. pombe*. This result was anticipated from the temperature-sensitivity of the original strains carrying the *rad4.116* mutation. Tetrad analysis of 6 asci exhibited a 0:2 segregation ratio for the disrupted locus, showing that the *rad4* gene is essential for spore germination, and, by extension, for mitotic growth.

DISCUSSION

Previous investigations on DNA repair in *S. pombe* have consisted of cellular, and in a few instances biochemical, studies on the properties of radiation-sensitive mutants which have been assigned to 23 different complementation groups. These studies, reviewed by Phipps *et al.* (4), have revealed some important differences between the repair pathways in *S. pombe* and *S. cerevisiae*. Wild-type *S. pombe* cells are somewhat more resistant to the lethal effects of UV-irradiation and considerably more resistant to ionizing radiation than *S. cerevisiae*. *S. pombe* lacks the photoreactivation pathway, but has a very efficient caffeine-insensitive excision-repair pathway for UV damage, which appears to have reduced activity in some of the mutants. On the basis of caffeine sensitivity, dimer excision and UV-mutability studies it is possible to assign tentatively the *rad5*, *rad10*, *rad11*, *rad13*, *rad15*, *rad16* and possibly *rad17* genes to the excision-repair pathway. There also appears to be one or more caffeine-sensitive pathways in *S. pombe*. Mutants deficient in these pathways (e.g. *rad1*, *rad3*, *rad4*, *rad8*, *rad9*) typically show sensitivity to both UV and γ -rays, and their sensitivity to UV is not potentiated by caffeine. UV-mutability in this latter group is reduced or unaltered when compared to wild-type cells. Recent data from our laboratory (23) have shown that the UV sensitivity of the *rad1*, *rad3*, *rad9* and *rad17* mutants results from a failure to arrest the cell cycle in G2 following UV-irradiation.

The *rad4.116* mutant, as well as being sensitive to UV and γ -radiation, is also thermosensitive for growth, and we have used this property to clone the *rad4* gene. This gene codes for a 579-amino acid neutral protein which possesses a possible zinc finger and a nuclear location signal. The 1.9Kb *rad4* gene transcript (Figure 4) is expressed at a very low level. Pulse field gel electrophoresis studies reported elsewhere (24) have shown that the gene is located on the 1.2 Mb *Not* I fragment D (25) on chromosome 1. The complete gene is able to correct both the temperature-sensitivity of the *rad4.116* mutant and its UV sensitivity (Figure 3), confirming that both these properties result from a single mutation (7). The first plasmid that we identified by its ability to complement the temperature sensitivity of the *rad4.116* mutant contained a truncated gene, missing the C-terminal 37 amino acids. When this truncated gene was retransformed into the *rad4.116* mutants, temperature-resistant transformants appeared, in spot tests, also to have acquired wild-type UV-resistance. When, however, a temperature-resistant transformant, was expanded, and full UV survival data obtained, the UV sensitivity varied considerably between that of wild-type cells and that of the *rad4.116* mutant (unpublished observations). The reason for this apparent discrepancy, and the overlap between the vital function and the DNA repair function of the *rad4* gene will be the subject of future experiments.

We have discovered that the N-terminal part of the *rad4* gene product has two regions of sequence similarity to the C-terminal

part of the human *XRCC1* gene, which was cloned by Thompson *et al.* (21) by virtue of its ability to complement a DNA repair defect in the Chinese hamster mutant EM9. This mutant is extremely sensitive to ethyl and methyl methanesulphonate (about 10-fold more sensitive than wild-type, based on D_{37} values), and slightly sensitive (about 2-fold more than wild-type) to ionizing radiation and UV light (26). The cells have a very high level of sister chromatid exchanges and a reduced rate of joining single-strand breaks following treatment with ionizing radiation or ethyl methanesulphonate (26). In contrast, the hypersensitivities of the *rad4* mutant to UV, γ -rays and EMS were similar for each mutagen (7). No biochemical characterisation of the defect in *rad4* mutants has been reported. The extent and position of the regions of sequence similarity do not permit us to propose that the *rad4* and *XRCC1* genes are truly homologous with similar functions in the two organisms. We suggest that the similar sequences represent important domains, such as an active site or area of protein-protein interactions.

The *S. pombe rad1* (27) and *rad9* genes (28) have recently been cloned. These genes have no homology with known DNA repair genes. We have also recently cloned the *S. pombe rad2*, *rad7*, *rad8*, *rad9*, *rad11*, *rad13*, *rad15*, *rad16*, *rad17*, *rad18*, *rad19* and *rad20* genes. Characterisation of these DNA repair genes will provide valuable information on the relationship of DNA repair in *S. pombe* to that in other organisms and should provide methods to isolate DNA repair genes from higher organisms.

ACKNOWLEDGEMENTS

We would like to thank Dr. A. Nasim, for supplying the *S. pombe* cell strains, Dr. J. Brannigan for the oligonucleotides, and A. Skidmore for carrying out the Northern blotting. This work was supported in part by EC Contract B16-E-142-UK and MRC project grant G88/03213CB. M. Fenech held an IARC Fellowship during part of this work.

REFERENCES

- Lehmann, A. R., and Dean, S. W. (1989) In Cooper, C. S. and Grover P. L. (eds.), *Handbook of Experimental Pharmacology*. Springer, Berlin. Vol 94 (II), pp. 71–101.
- Friedberg, E. C. (1985) *DNA Repair*. Freeman, San Francisco.
- Friedberg, E. C. (1988) *Microbiological reviews* **52**, 70–102.
- Phipps, J., Nasim, A., and Miller, D. R. (1985) *Adv. Genet.* **23**, 1–72.
- Lieberman, H. B., Riley, R., and Martel, M. (1989) *Mol. Gen. Genet.* **218**, 554–558.
- Gentner, N. E., Werner, M. M., Hannan, M. A., and Nasim, A. (1978) *Mol. Gen. Genet.* **167**, 43–49.
- Duck, P., Nasim, A., and James, A. P. (1976) *J. Bact.* **128**, 536–539.
- Gutz, H., Heslot, H., Leupold, U., and Loprieno, N. (1974) In King, R. C. (ed.), *Handbook of Genetics*, Plenum Press, New York, Vol. I, pp. 395–446.
- Nurse, P. (1975) *Nature* **256**, 547–551.
- Beach, D. B., Piper, M., and Nurse, P. (1982) *Mol. Gen. Genet.* **187**, 326–329.
- Carr, A., MacNeil, S. A., Hayles, J., and Nurse, P. (1989) *Mol. Gen. Genet.* **218**, 41–49.
- Wright, A., Maundrell, K., Heyer, W.-D., Beach, D., and Nurse, P. (1986) *Plasmid* **15**, 156–158.
- Hanahan, D. (1985) In Glover, D. M. (ed.), *DNA cloning—a practical approach*, IRL Press, Oxford and Washington. vol. I, pp. 109–135.
- Beach, D., and Nurse, P. (1981) *Nature* **290**, 140–142.
- Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Aves, S., Durkacz, B. W., Carr, A., and Nurse, P. (1985) *EMBO J.* **4**, 457–463.
- Durkacz, B., Carr, A., and Nurse, P. (1986) *EMBO J.* **5**, 369–373.
- Dorado, G., Steingrimsdottir, H., Arlett, C. F., and Lehmann, A. R. (1991) *J. Mol. Biol.* **217**, 217–222.
- Henikoff, S. (1984) *Gene* **28**, 351–359.
- Russell, P. (1989) In Nasim, A. Young, P. Johnson, B. F. (eds.), *Molecular Biology of the Fission Yeast*, Academic Press. Inc. pp. 243–271.
- Thompson, L. H., Brookman, K. W., Jones, N. J., Allen, S. A., and Carrano, A. V. (1990) *Mol. Cell. Biol.* **10**, 6160–6171.
- Grimm, C., Kohli, J., Murray, J., and Maundrell, K. (1988) *Mol. Gen. Genet.* **215**, 81–86.
- Al-Khodairy, F., and Carr, A. M. (1992) *EMBO J.* in press.
- Broughton, B. C., Barbet, N., Murray, J., Watts, F. Z., Koken, M. H. M., Lehmann, A. R., and Carr, A. M. (1991) *Mol. Gen. Genet.* **228**, 470–472.
- Fan, J.-B., Chikashige, Y., Smith, C. L., Niwa, O., Yanagida, M., and Cantor, R. C. (1988) *Nucl. Acids Res.* **17**, 2801–2818.
- Thompson, L. H., Brookman, K. W., Dillehay, L. E., Carrano, A. V., Mazrimas, J. A., Mooney, C. L., and Minkler, J. L. (1982) *Mutation Res.* **95**, 427–440.
- Sunnerhagen, P., Seaton, B. L., Nasim, A., and Subramani, S. (1990) *Mol. Cell. Biol.* **10**, 3750–3760.
- Murray, J. M., Carr, A. M., Lehmann, A. R., and Watts, F. Z. (1991) *Nucleic Acids Res.*, **19**, 3525–3531.